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Use of a colon simulation technique to assess the effect of live yeast on fermentation parameters and microbiota of the colon of pig

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ABSTRACT: The impact of 2 doses of a *Saccharomyces cerevisiae* were evaluated, 5×10^{10} cfu/kg of feed (L1) and 5×10^{11} cfu/kg of feed (L2) against a control (CON) with no added yeast, using an in vitro model [colon simulation technique (Cositec)] to mimic digestion in the pig colon. The L2 (but not L1) dose significantly improved DM digestibility compared to CON (61 v 58%) and increased NH_3 concentrations (+15%). Volatile fatty acid concentrations increased with L2 compared to CON—isobutyrate (+13.5%), propionate (+8.5%), isovalerate (+17.8%), and valerate (+25%)—but only valerate was increased with L1 (+14.2%). The analysis of microbiota from the liquid associated bacteria (LAB) and solid associated bacteria (SAB) revealed an interaction between the fraction and treatment ($P < 0.05$). Indeed, L2 had a significant impact on SAB and LAB ($P < 0.01$) whereas L1 only tended to change the structure of the population in the SAB ($P < 0.1$). Overall, this study showed that a live yeast probiotic could improve digestion in a colonic simulation model but only at the higher dose used and this effect was associated with a shift in the bacterial population therein.

Key words: Cositec, dry matter digestibility, microbiota, yeast.

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INTRODUCTION

Probiotics have been shown to improve weight gain and feed conversion and to reduce diarrhea and mortality in piglets. The inclusion of yeast supplements in pig diets improved performance (Shen et al., 2009) and reduced susceptibility to infection (Price et al., 2010). Performance gains with yeast supplementation may be related to modification of the intestinal microbiota composition and activity. The colonic microbiota has an important role in the gastrointestinal tract of pigs, providing energy to host (Cummings and Englyst, 1987) and improving resistance to colonization by pathogens (Hopkins and Macfarlane, 2003). Here we use an in vitro semicontinuous colonic simulation technique (**Cositec**) to investigate the effect of live yeast on digestive processes in the hindgut of finishing pigs.

MATERIALS AND METHODS

Cecal and proximal colon fluid (ascending and transverse colon) were collected from finishing pigs of about 100 kg (for both the inoculum and freeze-dried feed) fed a typical barley (*Hordeum vulgare*)-based finishing diet (circa 80% barley) that did not contain live yeast (collected at slaughter) and the system was run for 10 days (8 days adaptation and 2 measurement days) (Von Heimendahl et al., 2010). At the beginning of the experiment, 800 mL of gauze-filtered cecal and/or colon fluid were introduced into each fermentation vessel containing a perforated inner container filled with 2 nylon bags (50-μm mesh size), one introducing the fresh solid inoculum on day 1 and the second supplying 2.2 g of freeze-dried cecal and/or colon particles. On day 2, the nylon bag with the inoculum of the previous day was replaced with a nylon bag containing freeze-dried cecal and/or colon particles. The next day, the bags were changed alternately every 24 h to achieve a particle retention time of 48 h and a liquid dilution rate of 3%/h. Two doses of a commercial live yeast probiotic (*Saccharomyces cerevisiae* Sc47 at

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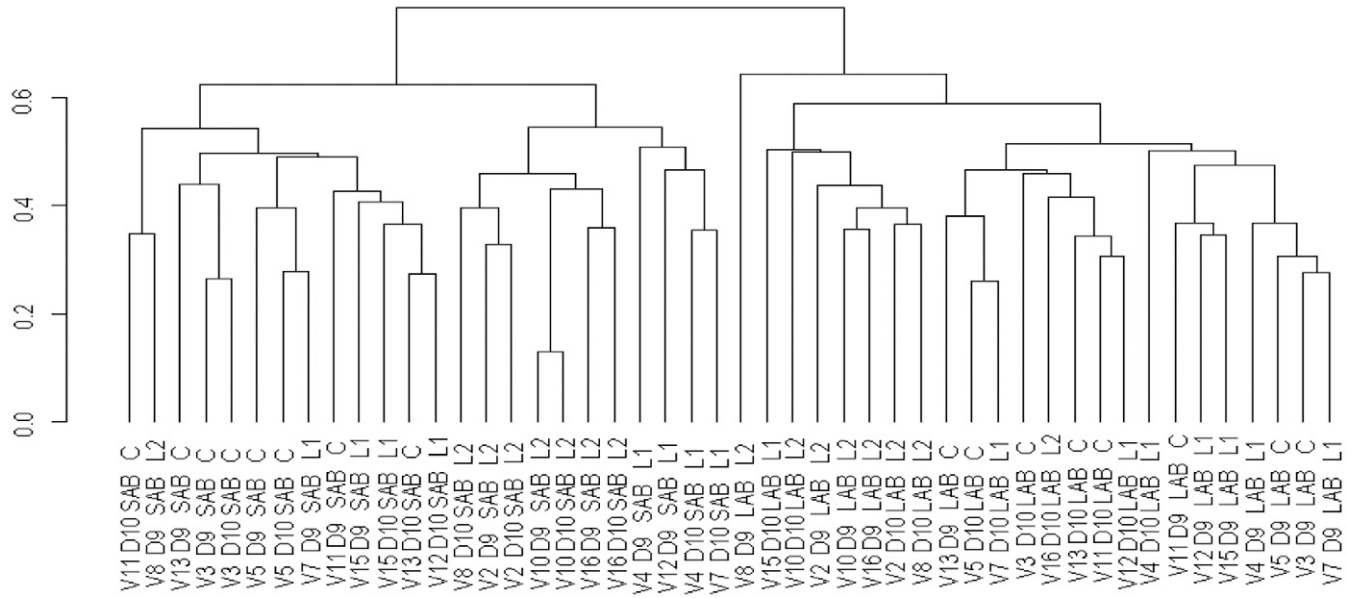

Figure 1. Complete linkage dendrogram of the Bray-Curtis distance. V = vessel number, D = day of sampling, LAB = liquid associated bacteria, SAB = solid associated bacteria, C = control, L1 = 5×10^{10} cfu/kg of feed, L2 = 5×10^{11} cfu/kg of feed.

10^{10} cfu/g; Lesaffre Feed Additives, Marcq-en-Baroeul, France) were used: 5×10^{10} cfu/kg of feed (**L1**) and 5×10^{11} cfu/kg of feed (**L2**) against a control (**CON**) with no yeast (n = 4). The pH, redox potential (Eh), and volume of total gas was recorded every day. During the measurement period, VFA and NH_3 concentrations were analyzed before and 2, 4, and 8 h after feeding using gas chromatography and/or flame ionization detector for VFA (Isac et al., 1994) and phenol-alkaline hypochlorite method for NH_3 (Parsons et al., 1984). Microbiota was analyzed by terminal RFLP adapted from Coolen et al. (2005). The DNA from the solid associated bacteria (**SAB**; collected from the washed 48-h incubated feed bag) and liquid associated bacteria (**LAB**) were extracted with the QIAamp DNA Stool Mini Kit (Qiagen Ltd., West Sussex, England). Genomic DNA was subjected to PCR with the primer combination 27F and 1389R and then digested with 4 restriction enzymes (*Hae*III, *Msp*I, *Hha*I, and *Tsp*509I; New England Biolabs, Hitchin, UK). All statistical analyses were performed with R statistical software (version 2.14.1). Physico-chemical parameters were analyzed using a randomized block design and repeated measures ANOVA and means were compared with Duncan's test ($Yij = \mu + \pi i + \tau j + eij$, in which Y = the dependent variable, μ = grand mean, πi = individual difference component for subject i, ej = effect of time, and eij = error for subject i and time j). Cluster analysis of terminal restriction fragment profiles was performed as well as multivariate analysis of variance (MANOVA) on the Hellinger transformed dataset (square root of the relative abundance of each peak).

RESULTS AND DISCUSSION

For pH, Eh, and gas production, no significant differences were found between treatments. The pH was maintained at 7.08 ± 0.2 and Eh was –250 ± 6 mV over the sampling period, similar to previous observations in Cositec (Von Heimendahl et al., 2010). Gas production decreased sharply between day 1 (1.1 ± 0.3 L) and day 2 but then remained fairly constant from days 2 to 10 (mean volumes 0.53 ± 0.1 L/day). Gas production is an indicator of an active fermentation (Pell and Schofield, 1993) and stability in gas production from day 2 to day 10 might suggest a stability in the fermentation and the adaptation of the microbiota within the Cositec. At the steady state, the yeast survival rate was 1% (results not shown), which is in accordance with previous studies (rat model) showing that the major loss of *S. cerevisiae* happened in the large intestine rather than in the stomach and small intestine (Garrait et al., 2007). There was an

Table 1. Mean VFA concentration and NH_3 over 4 sampling times (0, 2, 4, and 8 h).

	Treatment[1]			SEM	*P*-values
VFA (mM)	CON	L1	L2		
Acetic	7.79	7.65	8.24	0.127	<0.1
Propionic	3.51[b]	3.52[b]	3.81[a]	0.056	<0.05
Butyric	1.69	1.71	1.73	0.028	>0.05
Valeric	0.28[c]	0.32[b]	0.35[a]	0.006	<0.001
Isobutyric	0.37[b]	0.38[b]	0.42[a]	0.007	<0.001
Isovaleric	0.45[b]	0.46[b]	0.53[a]	0.010	<0.01
NH_3, mg/L	52.03[b]	52.59[b]	59.83[a]	0.678	<0.001

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Treatments: CON = control; L1 = 5×10^{10} cfu/kg of feed; L2 = 5×10^{11} cfu/kg of feed.

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improvement in dry matter digestibility (DMD) with L2 (61%) but no effect of L1 (57%) compared to the control (58%) ($P < 0.05$). The freeze-dried cecal and/or colon content used as feed for the fermentation contained a large proportion of fiber and protein (NDF = 412.4 g/kg and CP = 226.31 g/kg). The increased DMD may have been due to improved fiber or protein digestibility or both. Shen et al. (2009) used low levels of yeast inclusion in the diet of weaning pigs (5 g/d) and showed improved digestibility, daily gain, and live weight at slaughter. The microbiota tend to be in a transient state at this age (Pieper et al., 2006). Possibly in our experiment the microbiota may have been relatively stable due to the age of pigs and therefore a relatively large dose of yeast was required to initiate a shift in the microbiota. No significant interaction between treatment and time of sampling was observed for NH_3 or VFA measures. The peak of NH_3 concentration appeared 2 h after feeding ($P < 0.001$) and increased with L2 ($P < 0.001$) but no effect of L1 was recorded (Table 1). The increase of NH_3 production may reflect an improvement in protein digestibility but might also raise concerns as high levels of NH_3 from pig rearing units could be environmentally damaging (Philippe et al., 2011). However, an improved protein digestibility might also lead to a reduction of crude protein needed in the diet that could lead to the decreased incidence of postweaning diarrhea in weaned piglets (Kim et al., 2011). Volatile fatty acid concentrations peaked 2 h after feeding ($P < 0.001$) whereas isobutyrate and isovalerate peaked at 4 h ($P < 0.001$). Most VFA levels increased with L2 whereas only valerate increased with L1 (Table 1). Oeztuerk (2009) also found that acetate, butyrate, propionate, and isovalerate all increased in rumen simulation technique with the inclusion of live yeast in the diet. Cluster analysis and MANOVA revealed that the type of fraction (LAB vs. SAB) was the most important discriminative factor for the microbiota ($P < 0.001$) (Figure 1). Significant effects were found with L2 in both the liquid and solid fractions with clear modifications in the microbial population ($P < 0.01$). The MANOVA revealed that there was a significant interaction between fraction and treatment ($P < 0.05$). Indeed, no differences were observed in the structure of the microbiota between CON and L1 in the liquid fraction but there was a tendency in the solid fraction ($P < 0.1$) suggesting that live yeast would have a greater impact on SAB. In summary, the improvement in DMD with the higher level of yeast is reflected by an increase in VFA and NH_3 concentration, which appeared to be mediated through a shift in the microbiota and especially the solid associated bacteria.

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